IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

	Atty. Docket:	SOLOMON-1R
In re Application of:	Conf. No.:	3910
Beka SOLOMON	Art Unit:	1647
Appln. No.: 09/441,140	Examiner:	C. Nichols
Filed: November 16, 1999	Washington, D.C.	
For: PREVENTION OF PROTEIN AGGREGATION		

DECLARATION

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
2011 South Clark Place
Customer Window, Mail Stop
Crystal Plaza Two, Lobby, Room 1B03
Arlington, VA 22202

Sir:

I, the undersigned Beka Solomon, hereby declare and state as follows.

I am the same Beka Solomon who is the inventor of the invention disclosed and claimed in the above-identified reissue application.

I have been informed that, in an official action in the above-identified reissue application, the examiner contended that monoclonal antibody AMY-33 did not exhibit a significant inhibitory effect on metal-induced amyloid aggregation. The examiner stated that both zinc and aluminum are known to be present in physiological conditions, and he

thus questioned the enablement of *in vivo* use of the claimed monoclonal antibodies.

In order to demonstrate that the data provided in the present application is predictive of *in vivo* utility, I further declare and state:

The experiments to which the examiner refers are those in Example 2 of the present specification, the results of which are shown in Fig. 7A of the present application, which experiments are also discussed in the paper Solomon et al., Proc Natl Acad. Sci. U.S.A., 93:452-55 (1996), the results of which are illustrated in Fig. 1 thereof.

Example 2 and Figure 7 of the application demonstrate that AMY-33 exhibits an inhibitory effect on heat-induced amyloid aggregation in the absence of zinc and aluminum. The temperature used in the assay to induce aggregation of beta-amyloid, i.e., 37°C, is a physiological temperature. As discussed in detail below, I have confirmed that this assay is predictive of *in vivo* utility.

Initially, it should be noted that the examiner appears to have misunderstood the assay in Example 2 when the examiner refers in the office action to "metal-induced" beta-amyloid aggregation. In fact, aggregation of beta-amyloid in the assay was induced using heat, i.e., 37°C. The assay was carried out under three conditions, (a) heat alone, (b) heat

in the presence of Zn^{++} and (c) heat in the presence of Al^{+++}. The assay does not employ "metal-induced" aggregation *per se* as apparently contended by the examiner.

In order to demonstrate that heat-induced aggregation in the absence of zinc and aluminum is the experimental protocol that most closely correlates with aggregation *in vivo*, I have now repeated the experiments described in Example 2 using the 10D5 antibody, as well as the AMY-33 antibody. The experimental protocol was as described in Example 2 of the present specification and the results are provided in the same units. These experiments were performed by me or under my direct supervision. The results are shown in the following table, the first line representing heat-induced aggregation in the absence of zinc and aluminum, the second line heat-induced aggregation in the presence of zinc, and the third line heat-induced aggregation in the presence of aluminum. These results are also shown in the form of a graph in the attached Figure.

ABP(-AB)	ABP(+AB 10D5)	ABP(+AB AMY33)
0.25	0.54	0.53
+Zn 0.18	0.14	0.13
+Al 0.10	0.15	0.23

It can be seen from the above results that antibody 10D5 is effective in inhibiting heat-induced aggregation in the absence of zinc and aluminum, but is not very effective in inhibiting heat-induced aggregation in the presence of zinc or aluminum. In this regard, the results are similar to the results shown for the AMY-33 antibody. The results for AMY-33 are consistent with the results reported in the above-identified reissue specification.

AMY-33 is a monoclonal antibody which was raised using amino acids 1-28 of beta-amyloid as an immunogen (see Stern et al, Am. J. Path. 134:973-978 (1989)), and, as shown in Example 2 and herein, to maintain the solubility of soluble beta-amyloid.

The 10D5 antibody is a known antibody that was also raised against residues 1-28 of the beta-amyloid peptide. See Hanan et al, *Amyloid: Int. J. Exp. Clin Invest.* 3:130-133 (1996), and, as shown herein, to maintain the solubility of soluble beta-amyloid.

The 10D5 antibody has been shown to reduce pathology in a mouse model of Alzheimer's disease (see Bard et al. Nature Medicine, 6:916-919 (2000)), and to cause clearance of plaques *in vivo* in a mouse model for Alzheimer's disease (see Bacskai et al, Nature Medicine, 7:369-372 (2001)). See also Bard et al, Proc Nat Acad Sci U.S.A., 100:2023-2028 (2003) and

DeMattos et al, Proc Nat Acad Sci U.S.A., 98:8850-8855 (2001), and particularly page 8854 of the latter, where it states:

> Further, other mAbs previously found to be effective at suppressing Aß deposition *in vivo* (m3D6 and m10D5) [citing Bard et al, Nature Medicine, 2001] are able to act as Aß sinks in our dialysis experiments (data not shown).

As noted above, the 10D5 antibody has been proven to be active *in vivo*, even though it has been shown *in vitro* not to be very effective against heat-induced aggregation in the presence of zinc or aluminum. However, the 10D5 antibody has been shown above to be very effective against heat-induced aggregation in the absence of zinc and aluminum. Thus, it is apparent that the results of the heat-induced aggregation assay in the absence of zinc and aluminum are the most relevant to predicting *in vivo* activity. Accordingly, it would be expected that additional antibodies which are raised using amino acids 1-28 of beta-amyloid as the immunogen, or which otherwise recognize an epitope within residues 1-28 of beta-amyloid, and which inhibit heat-induced aggregation in the absence of zinc and aluminum as set forth in the above-identified reissue application, like the AMY-33 antibody, would also be active *in vivo*, notwithstanding the results of the heat-induced aggregation assay in the presence of zinc or aluminum.

copies of the publications cited herein are attached hereto.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2/19/04
Date

Beka Solomon
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